Droplet Size Matters: Bioavailability of CBD Emulsions

Relevant for: CBD, cannabis, nanoemulsion, Litesizer, dynamic light scattering, DLS, electrophoretic light scattering, ELS, food and beverage industry

Legalization of medical and recreational CBD consumption has led to increased research and development in edible variants of CBD including beverages, gummies or other products for oral administration. CBD is a highly lipophilic molecule and thus can be difficult to formulate, particularly in water-based systems. Nanoemulsion delivery systems, which encapsulate lipophilic CBD molecules with amphiphilic surfactants help to solubilize CBD in aqueous environments to increase the bioavailability. Particle size analyses and zeta potential measurements are a critical component for research and quality control of CBD emulsions because droplet size and stability dramatically influences the bioavailability of the final product. The Litesizer 500 enables precise and straightforward dynamic light scattering (DLS) and electrophoretic light scattering (ELS) measurements to determine the particle size distribution and zeta potential of CBD emulsions.

1 Introduction

Legalization of medical and recreational consumption of CBD in several countries has opened new markets for non-inhaled CBD products. In addition to pharmaceutical and cosmetic applications, one of the greatest opportunities is the development of cannabinoid-infused food/beverage products for oral ingestion.

Cannabinoids are the main active compounds in cannabis. The most prevalent are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) (1). While THC is a psychoactive component and often used to treat pain, anxiety, nausea, and insomnia, CBD is non-psychoactive and often consumed for its anxiolytic, anti-epileptic, and anti-inflammatory properties (1).

Upon inhalation, pulmonary absorption causes immediate effects but is also associated with the uptake of toxins and carcinogens produced by combustion or vaporization of organic matter (2). Oral ingestion of CBD reduces health risks, and consumers benefit from a gradual release and long-lasting effects (2).

However, oral ingestion has its own challenges. Due to the hydrophobic nature of CBD, the bioavailability, the fraction of an administered dose of unchanged drug that reaches systemic circulation, is significantly reduced and may vary from person to person (2). As a result, the onset times for the desired effects are delayed, and determination of optimal dosing is challenging. There are multiple reasons for a reduction in bioavailability including an incomplete absorption by the gastrointestinal tract as well as extensive ‘first pass metabolism’ by liver enzymes (2). A wide range of concepts and methods have been developed to create edible CBD products with defined and predictable onset times and systemic dosing levels over several hours.

The basis for all ingestible CBD products are extracts, which are defined as any solid or oil-like substance that concentrates the plant trichomes or their cannabinoid- and terpene-containing secretions (1). Trichomes are surface outgrowths of the plant epidermal layer. Depending on the method used, extracts contain variable concentrations of terpenes, which affect the taste and aroma, solvent contaminants, and product purity. Thus, downstream refining may be necessary to ensure final product quality.

Liquid formulations are commonly used to deliver ‘edible’ CBD products. They are typically generated by solubilizing CBD extracts in oils such as coconut derived MCT (medium-chain triglyceride) oils and

hemp seed oils. To improve water solubility the oil phase has to be encapsulated and dispersed in an aqueous phase, resulting in a CBD emulsion.

1.1 Emulsions

Emulsions are mixtures of two or more liquids that are normally immiscible. They represent two-phase systems where both phases, dispersed and continuous, are liquids (Figure 1). For oil-in-water emulsions, the dispersed phase is oil-based, and the continuous phase is aqueous.

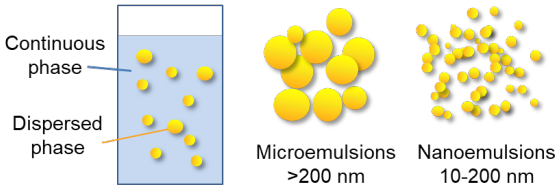

Figure 1: Characterization of Emulsions

Stable encapsulation of CBD in emulsions requires the use of surface-active molecules, e.g., surfactants/emulsifiers (2). If the proper surfactants are added to the formulation in optimal ratios, they decrease the interfacial tension between the oil and water phases, and enable emulsification.

Surfactant selection strongly depends on the emulsion properties and includes a wide range of amphiphilic proteins, polysaccharides, and phospholipids, as well as natural and synthetic small molecules. To increase CBD bioavailability, new methods for reducing the droplet size of the oil phase and creating micro- and nanoemulsions are being developed.

1.1.1 Microemulsions

The definition of a microemulsions is commonly given as a thermodynamically stable emulsion containing liquid droplets in the micro-scale range (diameters > 200 nm, Figure 1), which are stabilized by surfactants and co-surfactants.

Microemulsions form spontaneously without the need of shear forces to disperse the oil phase. Therefore, they are relatively easy to manufacture and have great potential for applications in pharmaceutical and cosmetic formulations as well as within the food industry (3).

1.1.2 Nanoemulsions

Nanoemulsions are oil-in-water or water-in-oil droplets emulsified by surfactant, co-surfactant, oil, and water with appropriate ratios. The criterion for an emulsion to be considered a nanoemulsion is for the droplet sizes to have characteristic diameters < 200 nm (Figure 1) (4). Each nano-droplet has a protective coating of emulsifier molecules that improve the solubility and bioavailability of CBD and bioactive components in general. Applications in the food and health industries benefit from common nanoemulsion characteristics such as small size, increased surface area, and increased stability. Preparation methods are distinguished between high-energy methods, such as high-pressure-homogenization, microfluidization, and ultrasonication, and low-energy methods such as phase-inversion emulsification and self-nanoemulsification (5). Research on CBD nanoemulsions has shown that systemic absorption of CBD is enhanced and onset times are shortened when compared to non-emulsified CBD oils (6).

1.1.3 Lipid based self-nanoemulsifying drug delivery systems (SNEDDS)

The principle of SNEDDS comprises a ‘preconcentrate’ of lipids, surfactants, and CBD that spontaneously assembles into nanoemulsions when the mixture reaches the gastrointestinal lumen. The lipophilic CBD is entrapped within a hydrophilic shell to increase water solubility. Bioavailability can be further enhanced by adding inhibitors of first pass metabolism enzymes, creating effective CBD products with predictable dosing levels (2).

1.2 Characterization of micro- and nano-emulsions with the Litesizer 500

Figure 2: Litesizer 500

CBD bioavailability strongly depends on the method of administration. For oral uptake, micro- and nanoemulsion droplet size is one of the most critical parameters and directly affects the pharmacokinetic and organoleptic properties. Therefore, droplet size requires optimization during formulation development, and particle size and zeta potential measurements are

useful tools in the quality control analysis of the final product.

Micro- and nanoemulsions are within the optimal size range for particle size analysis via dynamic light scattering (DLS). The Litesizer 500 enables DLS and electrophoretic light scattering (ELS) measurements using fast, non-invasive, and highly accurate and reproducible techniques. DLS measurements determine particle size distributions and ELS provides additional information about emulsion particle interfacial charge and stability via the zeta potential (7).

2 Experimental setup

Four independent CBD emulsion samples were analyzed via particle size distribution and zeta potential with the Litesizer 500 by ProVerde Laboratories, Milford, MA, USA. All measurements were performed in aqueous solutions at 25°C. For DLS disposable cuvettes were used and the input parameters: general for Analysis Mode as well as automatic settings for Measurement Angle, Optical Filter, and Focus mode were selected. ELS measurements were performed in the Omega cuvette. Transmittance values between 40% and 86% pointed out that the turbidity of the samples were in an ideal range for DLS and ELS measurements. All measurements were conducted in triplicate.

3 Results and Discussion

Bioavailability of emulsion-stabilized CBD strongly depends on the droplet size. The Litesizer 500 quickly and accurately determines very small droplets sizes in the nm-range up to 10 µm. Figure 3 shows the intensity-weighted particle size distributions of four CBD emulsions. The hydrodynamic diameters, mean particle sizes of the individual populations, and polydispersity indices are summarized in Table 1. The hydrodynamic diameter describes the particle size of all detected particles (oil droplets) as a single value. In case of a monomodal size distribution, the hydrodynamic diameter will have a similar value to the peak size of the main particle population. Samples 1 and 3 display monomodal distributions, with mean peak sizes of 44 nm and 275 nm, respectively. This indicates that sample 1 meets the definition for being a nanoemulsion, while sample 3 is a microemulsion. In contrast, samples 2 and 4 show polymodal particle size distributions. The primary population (mean peak 1) is in the nanoemulsion size range for sample 4 and in the microemulsion size range for sample 2. In both samples, a minor population is present in the smaller nm scale.

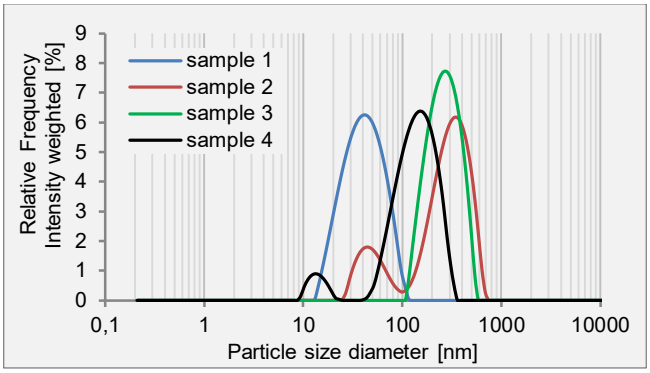

Figure 3: Intensity weighted particle size distribution of four CBD emulsion samples. Mean values of three consecutive measurements are displayed.

Sample	HDD [nm]	Mean particle size 1 [nm]	Mean particle size 2 [nm]	Poly-dispersity Index [%]
1	39.0 ± 0.1	44.0 ± 0.9	-	22 ± 1
2	223 ± 12	332 ± 9	50 ± 5	28 ± 3
3	257.9 ± 0.8	275 ± 6	-	16.7 ± 0.7
4	119.5 ± 0.6	151 ± 5	14.1 ± 0.4	24.8 ± 0.6

Table 1: Mean particle sizes and Polydispersity Index of CBD-emulsions measured with the Litesizer 500; mean±SD, n = 3, HDD = Hydrodynamic diameter

To achieve and maintain an optimal emulsion, CBD droplets need to be well dispersed and remain stable over time. Zeta potential measurements enable an estimate of the stability of CBD emulsions. Zeta potential values greater than 30 mV in magnitude are generally considered to be electrostatically stable. Figure 4 displays mean zeta potential distributions of the four CBD emulsions and Table 2 summarizes the mean zeta potential values. For sample 1, 2 and 3, the zeta potential values were greater than 30 mV in magnitude and are therefore considered to be stable. Sample 4 exhibits a lower zeta potential (-16.4 mV) indicating a potential instability that needs to be considered further. Depending on its composition, an emulsion with zeta potential less than 30 mV in magnitude is not necessarily unstable, as the emulsion may be sterically stabilized rather than electrostatically stabilized (8).

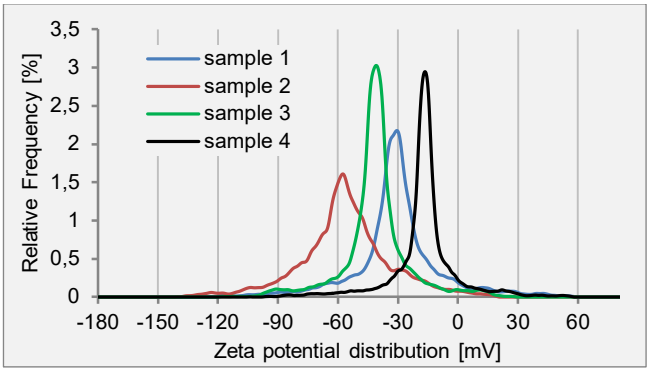

Figure 4: Zeta potential distributions of four CBD emulsions. Representative graphs out of three consecutive measurements of each sample are displayed.

sample	Mean zeta potential [mV]
1	-33.4 ± 0.5
2	-58.2 ± 2.4
3	-40.5 ± 0.8
4	-16.4 ± 0.2

Table 2: Mean zeta-potential values of four CBD emulsions. Mean ± SD of three consecutive measurements are listed.

4 Conclusion

Absorption, improved bioavailability in general and predictable dosing are key challenges that need to be analyzed and improved to create high-quality CBD emulsions. Particle size distribution and zeta potential are critical parameters in formulation development and quality control of the final product. This application report demonstrates that the Litesizer 500 is an easy-to-use tool for fast, reproducible, and accurate analyses of the particle size distribution and stability (zeta potential) of CBD emulsions. These parameters provide important information to improve and maintain product quality.

5 Acknowledgements

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6 References

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